

The University of Chicago

THE HISTOLOGICAL RESPONSES OF
IRESINE LINDENII TO INDOLE-
ACETIC ACID

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1937

By
BERTRAND FEREDAY HARRISON

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE BOTANICAL GAZETTE
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HISTOLOGICAL RESPONSES OF *IRELINE LINDENII* TO INDOLEACETIC ACID¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 484

BERTRAND F. HARRISON

(WITH NINETEEN FIGURES)

Introduction

Numerous studies of the gross responses of plants to applications of indoleacetic acid have been made in the past few years, but few studies have been concerned with the specific tissues involved. The responses of some of the tissues of *Coleus* stems treated with indoleacetic acid in lanolin were mentioned and pictured in the studies made by LAIBACH and FISCHNICH (7). FISCHNICH (4) found in treated *Coleus* stems that the cells of the parenchyma enlarge, the cells in the vicinity of the vascular bundles proliferate, the cambium becomes active, and new vascular bundles appear between the old ones. He found that the roots arise principally in the borders of the vascular bundles. CZAJA (3) investigated the effects of indoleacetic acid-lanolin paste on *Helianthus* hypocotyls, applying it as a ring encircling the hypocotyl, unilaterally, and apically on the cut surface of decapitated hypocotyls. Treated areas were sectioned and some of the tissue responses were described. Similar studies were made of *Pisum sativum*. SNOW (9) reported a definite stimulation of cambial activity in *Helianthus* hypocotyls treated with indoleacetic acid in gelatin. A detailed study of the histological responses of Red Kidney beans treated with this acid was made by KRAUS, BROWN, and HAMNER (6). Later this study was extended and the responses of beans to other growth promoting substances were also investigated (5). BORTHWICK, HAMNER, and PARKER (1) have studied the histological and microchemical reactions of tomato plants to indoleacetic acid.

The stems of bean and tomato each represent distinct anatomical patterns. Because *Iresine lindenii* Van Houtte has a pattern differ-

¹ Additional cost of publication sustained by the writer.

ing markedly from either of these two, it was chosen for the investigation here reported. This species evidently includes the garden forms *I. emersonii*, *I. collensia*, and *I. formosa*. It is a small erect herb belonging to the Amaranthaceae. Native to Ecuador, it is widely cultivated in this country as a bedding plant, owing to the bright red color of its leaves and stems.

This investigation was begun in July, 1936, and continued for a year. The plants were grown in the greenhouse under favorable conditions, but no attempt was made to provide constant conditions of light, temperature, or atmospheric humidity. Cuttings were started from four months to about a year before the time of treatment. No differences were noticed in the types of responses of the stems at different times of year, but there was a marked difference in the rate at which the reactions occurred. Reaction was rapid in warm moist weather and abundant sunlight.

The earlier experiments consisted in the treatment of cuttings with aqueous solutions of indoleacetic acid, then planting them in clean quartz sand both with and without bottom heat. In making up the solutions, a few milliliters of alcohol were used to dissolve the indoleacetic acid crystals before water was added. The control plants were treated with water containing an amount of alcohol equal to that in the indoleacetic acid solution.

The treatment of cuttings with aqueous solutions of indoleacetic acid stimulated the proliferation of some of the tissues and the production of numerous adventitious roots. It was found, however, that if the indoleacetic acid was applied to stems in a mixture of lanolin the tissue responses were localized in a much shorter portion of the stem. Since this method lends itself more readily to a histological analysis of the tissue responses, it was adopted for all subsequent experiments.

A mixture of 3% of Merck's crystalline indoleacetic acid in anhydrous lanolin was used in all cases. Two types of applications were made on each of two internodes of different degrees of maturity. The first obviously elongated internode down from the tip which had reached a length of 6-10 mm. is recorded as the first internode, and the second internode below the first is recorded as the third internode. One method of treatment consisted in encircling the

upper-middle portion of any given internode with a narrow band of the lanolin mixture, or of pure lanolin in the case of control plants (fig. 1A). Care was taken not to injure the stem otherwise. Not more than one internode was treated on any given stem. The other method of treatment consisted in decapitating the stem at the upper part of the first or third elongated internode, blotting the surface with absorbent paper, and applying the mixture or pure lanolin to the cut surface (fig. 1B, C).

Specimens were taken at various intervals, fixed in CROOKS modification (2) of Navashin's fluid, and imbedded in paraffin for sectioning. Sections were cut 10μ thick. The interpretations are based upon the examination of several thousand sections from plants of different ages and material in several stages of development.

Gross responses to treatment

AQUEOUS SOLUTIONS

At the beginning of this work a number of stem cuttings were treated with aqueous solutions of indoleacetic acid ranging in strength from 0.05 to 0.5 mg. per milliliter for fourteen hours. Even the weakest of these concentrations proved highly toxic. The average number of roots produced by the individual cuttings was in inverse proportion to the strength of the solution with which they were treated, the untreated controls showing the greatest number of roots. Subsequent trials showed that cuttings treated with a solution containing 0.1 mg. indoleacetic acid per milliliter of water for three and a half hours produced an average of about five times as many roots as did untreated controls, by the end of two weeks. Untreated stems produced roots only at the lowest node and within 2 or 3 mm. of the cut end of the internode. Treated plants frequently produced an abundance of roots the entire length of the lower internode.

INDOLEACETIC ACID-LANOLIN MIXTURE

First internodes which were treated with a ring of lanolin mixture began to swell soon after treatment. By the end of thirty hours the portion of the stem under the ring and adjacent to it had swelled noticeably and become somewhat lighter in color. During this time

the nearby leaves bent and twisted, although later they usually straightened out again. The stem may also bend and twist and may remain crooked. The treated area continued to increase in size slowly and rather uniformly for about ten days, after which there was little further enlargement. Meanwhile the stem, a short distance above and below the lanolin ring, formed a spindle-shaped tumor. The response was usually confined to a few millimeters on each side of the band, but sometimes the whole internode became involved. Roots penetrated the surface of the stem about ten days after treatment. They may continue to grow until they are about 4 or 5 mm. beyond the surface of the stem, when they usually dry up. If the atmosphere is humid they continue to elongate. Since there are numerous roots formed, the cortex may be partially lifted off or torn; the surface of the stem thus becomes rough and broken, or a number of longitudinal cracks may be formed. Previous to the appearance of roots the surface of the swelling is usually ridged, the ridges resulting from the enlargement of the collenchyma of the cortex.

Control plants in which the first internode was ringed with pure lanolin showed a very slight enlargement under the ring. No roots were formed nor did the swelling extend perceptibly beyond the limits of the lanolin.

Third internodes which were ringed with indoleacetic acid mixture showed very local swelling confined to a few millimeters in breadth. There is considerable difference in the state of maturity of these so-called third internodes, so there were some differences in the reactions of different stems. The less mature stems showed more obvious responses than more mature ones. The enlarged areas were not noticeably ridged nor broken except where roots penetrated the surface. Root primordia appeared in ten to twelve days. There were fewer roots formed than in the first internode; those which were formed behaved much as did those of the first internode. Not all of the roots formed penetrated to the outside; some grew downward just inward from the collenchyma of the cortex.

Control plants in which the third internode was ringed with pure lanolin showed almost no responses which could be detected with the unaided eye.

The first noticeable response of decapitated first internodes to applications of lanolin mixture to the cut surface was a slight swelling of the stem immediately below the treated surface about thirty hours after treatment. The stem continued to increase in diameter,



FIG. 1.—Stems of *Iresine* at beginning of experiments. A: lines indicate portion where indoleacetic acid in lanolin would be applied encircling first or third internode. B: decapitated first, and C: decapitated third internode with layer of lanolin applied to cut surface.

the greatest swelling taking place about 2 mm. below the cut surface. There was no development of tissue above the original level of the cut surface and less than the first centimeter of the stem was affected by the treatment. The surface of the swelling may be ridged, but usually it remained rather smooth.

The root primordia penetrated the epidermis just below the cut

surface about nine days after treatment. An almost complete ring of roots was usually formed, and in this area the cortex was lifted off the stem (fig. 3*A*). The dried fragments may remain attached to the edge of the intact cortex. As in laterally treated stems, the roots grew out from the surface 4-5 mm. and then dried up and

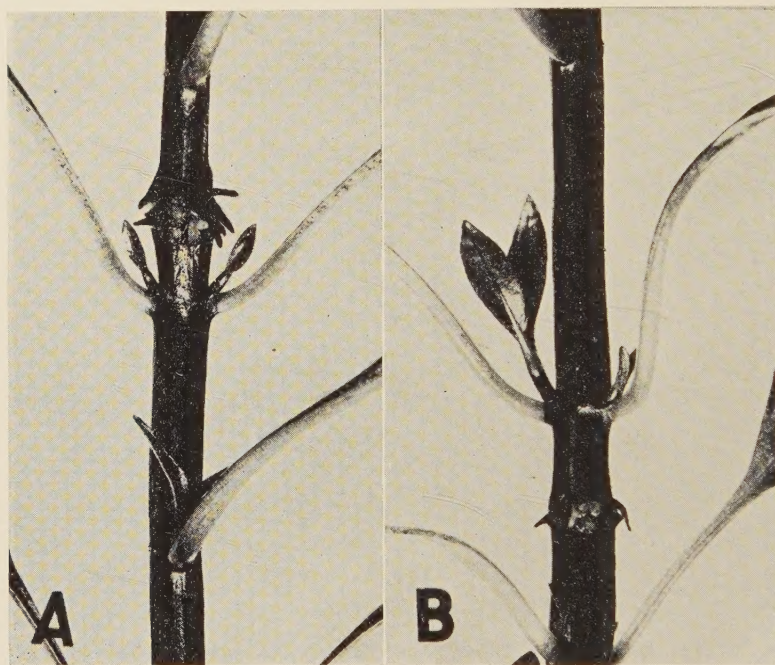


FIG. 2.—Stems 360 hours after ringing with lanolin mixture. *A*: first internode; *B*: third internode.

ceased to elongate unless kept in a moist atmosphere. A few stems were observed for about a month after treatment; after about fourteen days little change was noticed in their external appearance.

First internodes which were decapitated and pure lanolin applied to the cut surface showed a very slight swelling immediately adjacent to the cut surface. No subsequent changes were observed.

Decapitated third internodes treated with lanolin mixture showed essentially the same responses as did treated first internodes, except that the stems were much less reactive. The swellings were smaller in proportion to the size of the stem, and the number of roots pro-

duced was usually not so great. Within about thirty to forty hours the stem began to swell a few millimeters below the cut surface. This continued until the enlarged portion was about double the diameter of the original stem. There was little further change until the roots emerged after about twelve days (fig. 3*B*). The cortex was



FIG. 3.—Decapitated stems 312 hours after lanolin mixture applied to cut surface. *A*: first internode; *B*: third internode.

torn loose where the roots emerged and the roots reacted as in the other cases.

The decapitated third internodes which were treated with pure lanolin as controls showed no marked responses except slight enlargement immediately adjacent to the cut surface.

Histological details

NORMAL ANATOMY

Transections of the first internode of *Iresine* stems are somewhat oval in outline. Two prominent opposite ridges which extend the

length of the internode alternate with two lateral ridges. A group of three primary vascular bundles is located in each of these four ridges, and through part of the internode four additional bundles may alternate with these four groups, making a total of twelve to sixteen bundles (fig. 44). The bundles may anastomose near the node but they do not become united laterally to form a continuous cylinder of vascular tissue. WILSON (10) has described the anatomy of *Iresine paniculata*, and his description applies to this species as well. Two or three layers of collenchyma occur subepidermally in each of four ridges. Between the ridges the cells are thin walled and contain numerous chloroplasts. There are seven to ten layers of cortical cells but no well defined endodermis.

In young stems the pericycle appears as a nearly continuous band one cell in thickness. Divisions in a centripetal direction produce at intervals cells which mature to form small bundles of functional xylem. Between these bundles the cells formed by the pericycle and its derivatives become uniformly thickened, and at maturity appear nearly square in transection and rectangular in longitudinal view. WILSON calls this conjunctive tissue and attributes it not to a pericycle directly but to an extrafascicular cambium formed by the pericycle. After a small bundle of xylem cells has been formed, the derivatives of the pericycle external to the bundle function as a fascicular cambium and divide centrifugally to form a number of cells which mature as phloem. On each side of the bundle additional layers of conjunctive tissue are laid down. Later the bundle may become completely imbedded in this tissue. The number of layers of thickened cells varies with different plants for corresponding internodes and also with the level in any given internode. The tissues in the upper portion of an internode are the most nearly mature since the base of each internode remains relatively meristematic. In the older internodes the bundles inward from the conjunctive tissue may be separated from it by several layers of cells of primary origin. This condition is seen especially at the middle of the internode.

Adventitious roots develop from untreated cuttings within six to ten days after they are planted in moist sand. The roots arise from very near the cut surface at the base and from the internode

near the lower node or nodes if more than one node is buried in the sand. Scarcely any roots arise from the internodes of untreated stems except from that portion which is very near the cut surface or a node. The tissue from which the adventitious roots arise is the pericycle and its undifferentiated derivatives. The first divisions in the formation of the roots usually occur in the outer layer of cells; that is, in the pericycle, and soon afterward the next layers inward also become active. From the cells which arise from the first layer there is organized a calyptragen from which the epidermis and the root cap are developed. The histogens for both the periblem and the plerome appear to originate in cells derived from the second layer; that is, the one immediately underlying the pericycle. The layers of cells inward from these outer layers divide actively and form a number of cells which make up that part of the root nearest the center of the stem. Root primordia often arise external to the small secondary bundles imbedded in the conjunctive tissue, in which case the phloem of these bundles becomes active and is involved in the formation of the lateral roots.

The principal responses of the various tissues to treatment are illustrated by means of photomicrographs. These show some of the more obvious changes which were observed.

RESPONSES OF FIRST INTERNODES ENCIRCLED WITH LANOLIN MIXTURE

Following lateral applications of lanolin mixture to the first internodes, the cells of the epidermis become very reactive. Soon after treatment they enlarge, principally in a radial direction (fig. 4*B*), and after considerable expansion divide, mainly tangentially. In local areas the epidermal cells divide repeatedly, forming small protuberances of cells which project beyond the neighboring ones. By the end of 216 hours practically all the epidermal cells in the treated area had divided tangentially at least once (fig. 7*A*). Subsequently a new epidermis is organized from the outer layer of daughter cells.

The cortical collenchyma is the first tissue to show noticeable responses to lateral treatments. Within thirty hours the cells had begun to enlarge. Enlargement continues to such an extent that

the cells lose their characteristic appearance as collenchyma, the walls become distended, and the thickenings disappear. There are scarcely any intercellular spaces (figs. 4*B*, 5*A*). The cytoplasm of the cells becomes dense and many of the cells divide, mainly tangentially. Some of the cells respond much more readily than others, but divisions of the cells of the collenchyma are never numerous.

The cells of the cortex, exclusive of the collenchyma, show little response except a slight general enlargement. By the end of 168 hours there may be a slight increase in the number of divisions over that of untreated stems, especially in the inner cortical cells (fig. 7*A*). There is no well defined endodermis but such as is present shows only slight response. External to young root primordia the cells may divide a number of times, but ordinarily they are only slightly more active than the other cells of the cortex.

The first cells of the pericycle to respond are those which occur external to the vascular bundles (fig. 4*A*). Soon, however, the pericycle cells which lie interfascicularly also proliferate, and a continuous band of meristematic cells is established (fig. 6*B*). The cells of the meristematic layer are involved in the initiation of root primordia but to a lesser extent than the cells of either the phloem or rays.

Of all the first internode tissues the phloem is the most responsive to treatment. Beginning soon after treatment the cells enlarge in all directions, especially radially. At the outer periphery of the bundles a large cap of highly meristematic cells is formed from the phloem, pericycle, and in some cases from the cells of primary origin which lie between the phloem and the pericycle (fig. 5*A*). After the extrafascicular cambium becomes active its newly formed derivatives contribute to this mass of cells, which lie just inside it. The tissues exterior to this cap of cells are pushed outward and laterally. The sieve tube-companion cell groups become widely separated from one another, and some time after treatment they appear scattered throughout the mass of proliferating cells. The cells of this active tissue contribute to the formation of the adventitious roots.

While the fascicular cambium remains active following treatment with indoleacetic acid, it does not appear to be markedly stimulated. The cambial zone remains rather narrow, and as a result

most of the sieve tubes and companion cells do not become separated from the xylem by a very wide zone (fig. 7*A*, *B*). No divisions were observed in the cells of the xylem parenchyma although they may enlarge slightly.

The cells of the pith, except those of the rays, are affected little or not at all by lateral treatments. In a few stems some of the pith cells appeared slightly enlarged, but no other changes were observed in the pith at any level of the treated stem.

The ray tissue lying between the bundles and especially that immediately adjacent to the phloem responds more actively to treatment than any other tissue except the phloem. The ray cells are among the first to become active, and this activity spreads laterally until there is a band of meristematic cells between each pair of bundles (fig. 5*A*). This meristematic band becomes broader by subsequent divisions of the cells composing it, and by divisions of the pericyclic cells, until a broad zone of meristematic cells is formed extending from the level of the fascicular cambium outward to the endodermis. The endodermis is finally pushed outward, well beyond its former position (fig. 7*A*).

It is from this zone of meristematic cells both over and between the bundles that the primordia of the roots arise. By far the greater number of roots originate in the ray cells immediately adjacent to the phloem, with the phloem, rays, and pericycle each contributing to their formation (fig. 6*A*). Occasionally roots develop from the ray and pericycle about equidistant between two bundles (fig. 6*B*). In the formation of the roots from first internodes it seems that the histogen of the calyptra arises from the pericycle and that the periblem and plerome arise from derivatives of the rays.

As the roots increase in length they push their way outward through the cortex. No evidence of enzymatic digestion of the cortical cells was observed; instead the cells are crushed and pushed together ahead of the lengthening root. Not all of the roots reach the surface; some grow downward or laterally through the cortex just within the collenchyma.

Some of the derived cells which lie inward from the root tip differentiate as xylem elements and phloem elements, so that the

vascular system of the young root becomes directly connected with similar elements of the vascular bundles of the stem (fig. 7*A, B*).

First internodes which were encircled with pure lanolin as controls resembled the treated stems in the activity of the epidermis. Epidermal cells of control plants enlarged and divided periclinally in the region where lanolin was applied, and 384 hours after treatment the majority of the epidermal cells had divided at least once. The epidermis was but slightly less active than in stems treated with the lanolin mixture. In figure 5 a stem 144 hours after treatment with the mixture is compared with a stem 384 hours after treatment with pure lanolin. The cells of the collenchyma in stems treated with pure lanolin also enlarged slightly although no divisions were observed. Through a very short vertical distance the other cells of the cortex may also become slightly enlarged. In none of the other tissues were any differences from untreated stems observed.

RESPONSES OF DECAPITATED FIRST INTERNODES TO APICAL APPLICATIONS OF LANOLIN MIXTURE

The epidermal cells of apically treated first internodes do not respond as in laterally treated stems. Those changes which do occur are found largely within 1 mm. of the cut surface, where the majority of the epidermal cells enlarge to about one and one-half times their original size but show no further obvious responses. Those immediately below the cut surface, and some in vertical strips, may enlarge to more than double their original size and divide as in laterally treated stems. Since this response is local rather than general, it may be because of lanolin coming in contact with the side of the stem.

Soon after treatment the cells of the collenchyma begin to enlarge, principally in a radial direction; subsequently a few of them may divide. At first only the cells immediately adjacent to the cut surface enlarge, but later cells farther down become involved although the region of activity never extends more than a few millimeters down the stem. As with the epidermis, there may be considerable increase in the size of the cells and active divisions in localized vertical strips of the collenchyma.

The inner or parenchymatous cells of the cortex show little change

for the first 72 hours following treatment, but later they enlarge and some of them divide. Not all of the cells are equally affected; in local areas the cells may divide repeatedly in all directions while in other parts at the same level they may divide but rarely. All intermediate conditions exist. The activity of the cortical cells is confined largely to the first 2 mm. below the cut surface. In the root zone much of the cortex is torn from the stem by the developing roots. No special activity in the endodermis was observed.

The pericycle is one of the more reactive tissues of the stem. Soon after treatment the cells enlarge slightly, the cytoplasm becomes dense, the nuclei become more prominent, and directly thereafter some of the cells divide (fig. 8*A*). As in laterally treated stems, the pericycle becomes active first over the bundles and later between the bundles, so that a nearly continuous band of proliferating cells is formed.

The cells of the phloem are extremely reactive. They may enlarge to more than double their former size before they divide. Following division the daughter cells enlarge and divide repeatedly. At the same time the cells of the rays begin to proliferate (fig. 8*A*, *B*), repeated divisions occurring centripetally. This often results in a long row of cells all derived from a single parent cell (fig. 9*A*). The proliferating cells of the phloem, pericycle, and rays contribute to the formation of a broad zone of meristematic tissue which extends as a continuous band outward beyond the original limits of the vascular bundles (fig. 9*A*, *B*).

The root primordia are organized from this zone of meristematic cells. As in laterally treated stems, the primordia arise mainly from the ray tissue and the pericycle, although some of the phloem derivatives may also be involved. The roots are frequently so close together that almost the entire band of cells is involved in root production, and fasciation is not uncommon (figs. 9, 10*B*). As the roots develop, an increasing number of cells within this band become active. There were numerous divisions in the outer cells of the pith. The cells of the pith frequently divide actively throughout the first millimeter below the treated surface, but few divisions were observed in the pith below this level except at its periphery. No cases

were observed in which the pith had proliferated sufficiently to project above the level of the cut surface.

The proliferation of the phloem cells and of the cambium derivatives forces the sieve tube-companion cell groups outward and separates them into smaller and smaller groups. The cells of the xylem parenchyma proliferate and spread the elements of the xylem apart. At the same time many of the ray cells differentiate as tracheids more or less at random (fig. 10C). These activities result in a nearly continuous band throughout which vascular elements are distributed, whereas the bundles of laterally treated stems remain sharply separated (fig. 10A, B).

The responses of decapitated first internodes to apical applications of pure lanolin consisted principally in a slight enlargement of cells of the cortex and pith near the cut surface. There was also some enlargement and radial division of some of the cells of the epidermis and collenchyma. In stems taken 384 hours after treatment these responses were confined to a vertical distance of less than 500 μ from the cut surface. All other tissues appeared as in untreated stems.

RESPONSES OF THIRD INTERNODES ENCIRCLED WITH LANOLIN MIXTURE

At the time of treatment of the third internodes, the pericycle and its derivatives appear as a continuous ring four to eight layers of cells thick, surrounding the stele. There is a marked difference in the state of maturity of these cells for different plants and at different levels in any given internode. In some third internodes none of these cells has any noticeable thickenings of the walls, while in others there are three or four layers of conjunctive cells with heavily thickened walls, and outward from these are three or four more layers in which the walls are not thickened. The responses of this latter type to treatment differ markedly from those of first internodes, but third internodes of the first type resemble more closely first internodes in their responses.

The tissues of the third internode, especially the more mature internodes, are much less responsive to treatment than those of first internodes. The epidermal cells of treated third internodes enlarge only slightly beyond their original size. Subsequently they

divide in regular tangential divisions with little further increase in size (figs. 12*B*, 14). The cells of the collenchyma respond much as in laterally treated first internodes but to a lesser extent. The cells enlarge about one and one-half times, principally in a radial direction. A small proportion of them divide.

The cortex, exclusive of the endodermis, shows but slight enlargement of the cells. The endodermis is reactive to the extent that there are occasional divisions, especially in the vicinity of developing roots.

The pericycle and its derivatives, together with the phloem of the secondary bundles, are the most responsive tissues. In stems in which the walls of the conjunctive cells have not become thickened, the entire band of conjunctive tissue, or nearly all of it, becomes meristematic and the tissues interior to it may also become involved (fig. 13*A*, *B*). The tissues inward from the conjunctive tissue which become active are the phloem, ray cells, and a cap of cells of primary origin which occur between the primary bundle and the conjunctive tissue. Cells of the pith are not noticeably affected.

In stems which have a number of layers of thickened conjunctive cells the responses to lateral treatments are confined to the non-thickened conjunctive cells and those external to them. Soon after treatment, the phloem of the secondary bundles and the nearby pericycle and its unthickened derivatives become active. Only rarely do any of the conjunctive cells with thickened walls enlarge or divide. Usually the first cells to respond are those which lie directly external to the primary bundles (fig. 12*A*, *B*). Later the remainder of the pericycle and its derivatives may proliferate, but it is in the vicinity of the primary bundles that most of the roots arise. Apparently the calyptrogen develops from the pericycle and the periblem arises from the younger pericyclic derivatives which are still meristematic. The plerome seems to develop either from the young pericyclic derivatives or from the phloem of the secondary bundles (fig. 14).

As the lateral roots increase in length, some of the cells derived from the root tip differentiate as vascular elements. In stems in which there is little or no thickened conjunctive tissue, the cells lying inward from the root (including those of the conjunctive tissue)

may also differentiate as vascular elements and become part of the conductive system of the root. In such cases the xylem elements and the phloem elements of the young root subsequently extend through the band of conjunctive tissue and become connected with similar elements in the primary vascular bundles of the stem, although they may also become connected with the secondary bundles occurring in the conjunctive tissue (fig. 13*B*). In contrast to the condition just described, the vascular systems of roots which develop outside of a number of rows of thickened conjunctive cells do not become connected with the elements of the primary vascular bundles. Cells derived from the root tip differentiate as xylem and phloem elements adjacent to similar elements of the secondary vascular bundles, which lie in the band of conjunctive tissue (fig. 15).

Third internodes which were encircled with pure lanolin as controls differed only slightly from untreated stems. Most of the epidermal cells under the ring of lanolin became slightly enlarged and divided tangentially. A few of the epidermal cells 384 hours after treatment had divided several times. None of the other tissues in the control plants showed any noticeable differences from untreated stems.

RESPONSES OF DECAPITATED THIRD INTERNODES TO APICAL APPLICATIONS OF LANOLIN MIXTURE

Third internodes which were treated apically proved to be very reactive. They are not so reactive as first internodes but they show much greater activity than third internodes treated laterally. All tissues made up of living cells responded to some extent to apical treatments with the lanolin mixture.

Near the cut surface of the apex the cells of both the epidermis and the collenchyma become enlarged, although few of the cells divide. At distances of more than 1 mm. from the apex the cells of both tissues show no obvious differences from those of untreated stems. The cells of the cortex in small local areas show numerous divisions, but there is no general proliferation and such activity as does occur is confined to a very short vertical distance from the treated surface. As in the other treatments, the cells of the endoder-

mis may show a limited number of divisions but they do not become markedly meristematic.

The reactions of the other tissues are more or less a combination of the reactions manifested by laterally treated third internodes and apically treated first internodes. The pericycle and its derivatives begin to proliferate soon after treatment (fig. 16*A*). In most of the stems examined there was a rather general proliferation of this tissue throughout its whole circumference. Root primordia arise from the pericycle, the pericyclic derivatives, and the phloem of the secondary bundles as in laterally treated third internodes, but root primordia also arise from the pericycle and its derivatives where there is no phloem, especially in stems which have very little thickened conjunctive tissue (fig. 17*A*). Numerous root primordia were produced. More of the cells of the conjunctive tissue became active than in laterally treated third internodes.

All of the conjunctive cells with thin walls became active and proliferated extensively, and many of the conjunctive cells with thickened walls also divided extensively. The cells with thickened walls which did not divide were left as small isolated groups surrounded by proliferating tissue. Many of the conjunctive cells with thickened walls became differentiated as xylem elements with irregular wall thickenings. Some of the proliferating conjunctive cells differentiated as vascular elements which served to connect the vascular system of the young lateral roots with similar elements in the primary bundles. The vascular systems of a large majority of the lateral roots formed became connected with those of the primary bundles as described for laterally treated third internodes, but here also the vascular system of the young root may become connected with similar elements in the secondary bundles, at least in the case of the xylem. In the older stem sections examined there was considerable new xylem differentiated in the primary bundles from cells derived from the fascicular cambium. The xylem of many of the young roots became connected with this new xylem in the primary bundles. Some connections between the xylem of the secondary bundles and that of the primary bundles were established.

In contrast to the conditions found in laterally treated stems, those treated apically showed great activity in the tissues which lie

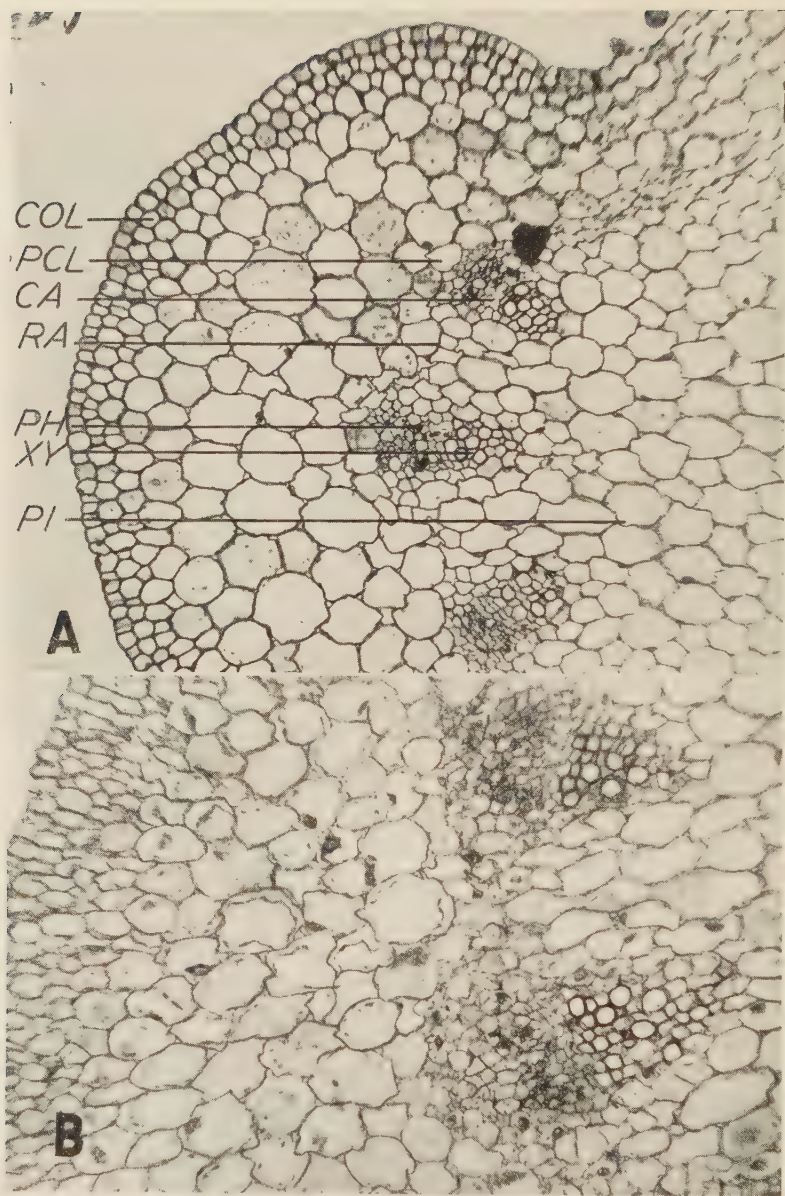


FIG. 4.—*A*: transection of first internode showing nature of tissues at beginning of experiments (*col*, collenchyma; *pcl*, pericycle; *ph*, phloem; *ca*, cambium; *xy*, xylem; *ra*, ray; *pi*, pith). *B*: first internode 110 hours after lateral treatment. Epidermis, collenchyma, and cortical parenchyma cells enlarged. Pericycle over bundles, phloem, cambium derivatives, and ray cells, especially those adjacent to bundles enlarged and dividing. Some xylem parenchyma cells enlarged slightly.

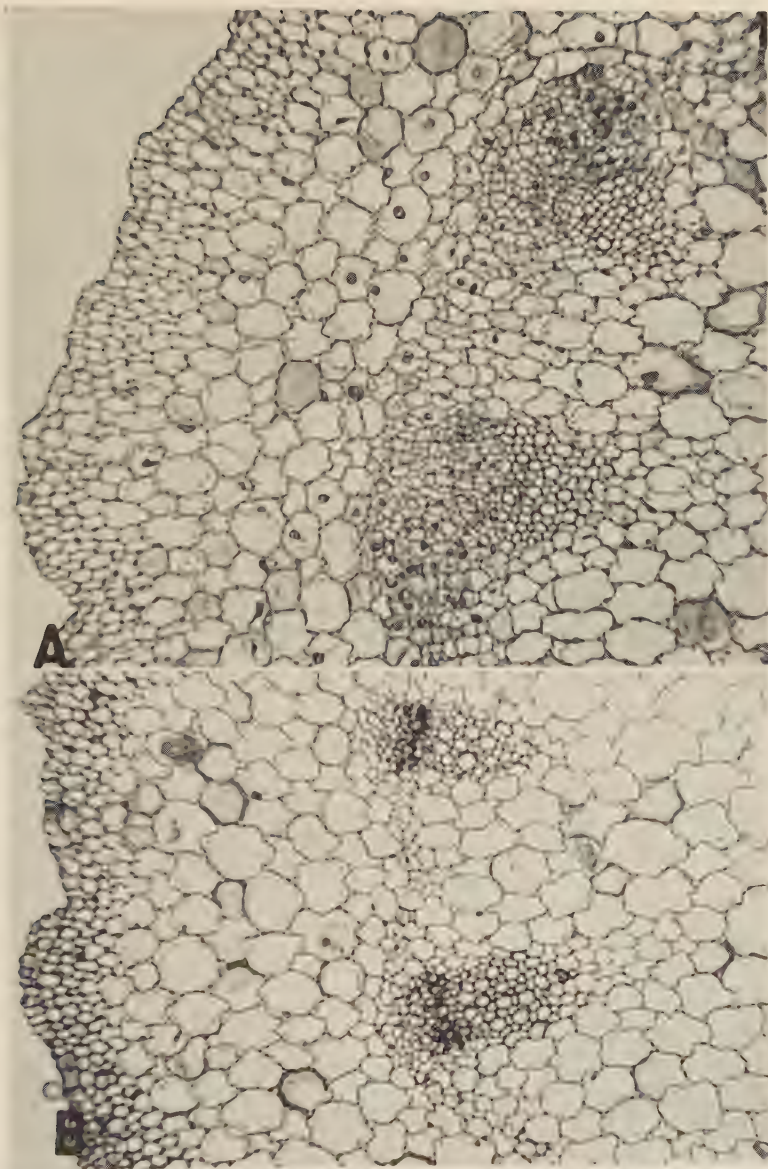


FIG. 5.—*A*: first internode 144 hours after lateral treatment. Phloem, pericycle, and ray tissue show increased activity. Root primordia have begun to be organized from ray tissue adjacent to each bundle. *B*: transection of control stem 384 hours after being ringed with pure lanolin. Epidermal cells have enlarged and divided tangentially; cells of cortex, especially those of collenchyma, have enlarged radially; other tissues as in normal stem of this age.

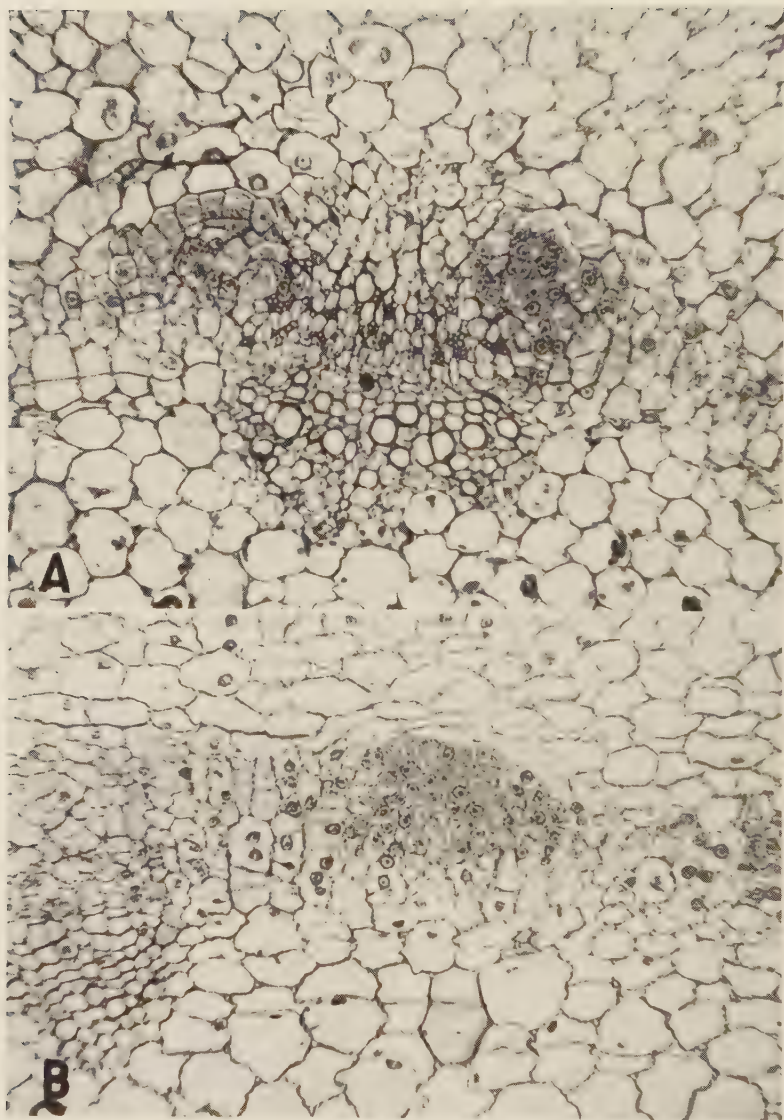


FIG. 6.—*A*: laterally treated first internode 144 hours after treatment, showing two root primordia developed from ray tissue and phloem of adjacent bundle. Pericycle and phloem of bundle active. *B*: origin of root from tissues between bundles; pericycle and ray tissue involved; 168 hours after treatment.

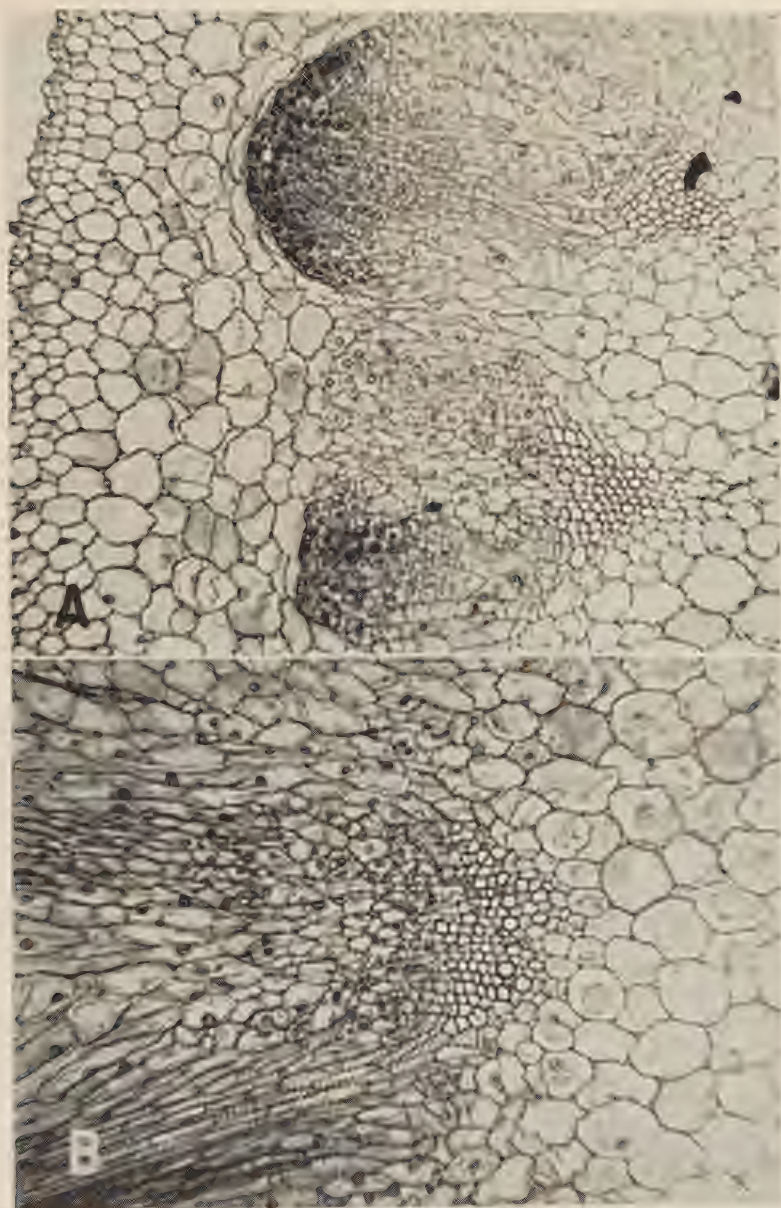


FIG. 7.—*A*: development of roots in stem 216 hours after treatment. Vascular tissue of root has differentiated back to that of bundle. Epidermal cells have divided tangentially. Phloem and ray cells have proliferated extensively. *B*: details of reactions 312 hours after treatment. Phloem and ray cells show great activity. Connection of vascular elements of root with that of stem is shown. Xylem parenchyma active; pith not meristematic.

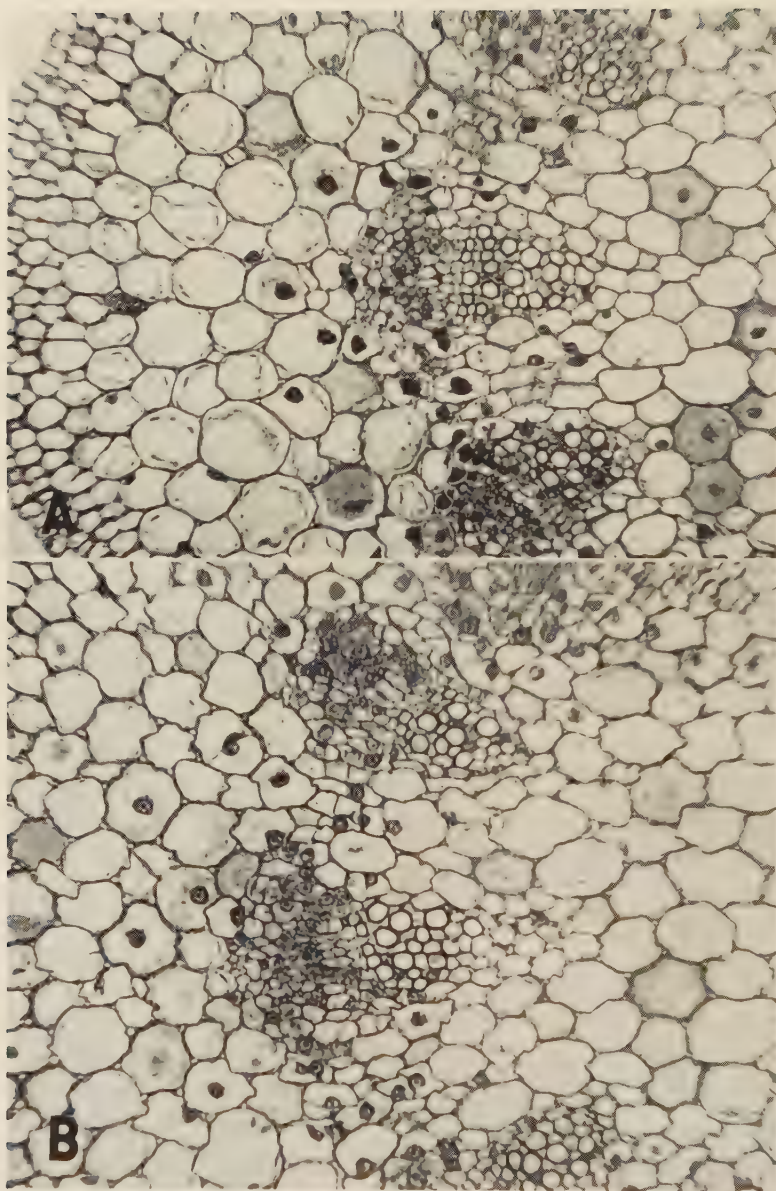


FIG. 8.—*A*: first internode 80 hours after indoleacetic acid was applied to apical cut surface. Pericycle, phloem, cambium derivatives, and ray cells show beginning of some activity. Epidermal and collenchyma cells show slight radial enlargement. *B*: 93 hours after treatment. Pericycle, phloem, and ray cells have begun to divide.

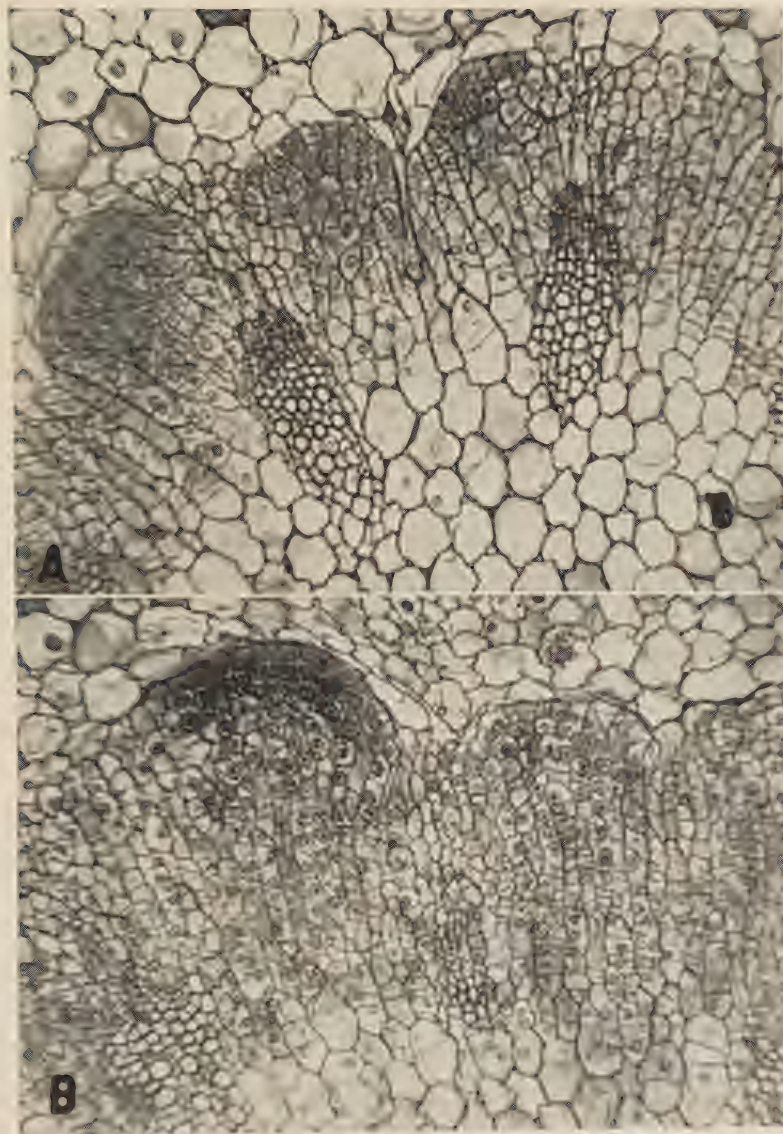


FIG. 9.—*A*: internode 144 hours after treatment. Root primordia organized from pericycle, phloem, and ray tissue; ray tissue especially reactive; xylem parenchyma slightly active. *B*: condition similar to *A* 168 hours after treatment. Ray tissue proliferated to form a nearly continuous band; activity extends farther into pith; phloem largely meristematic.

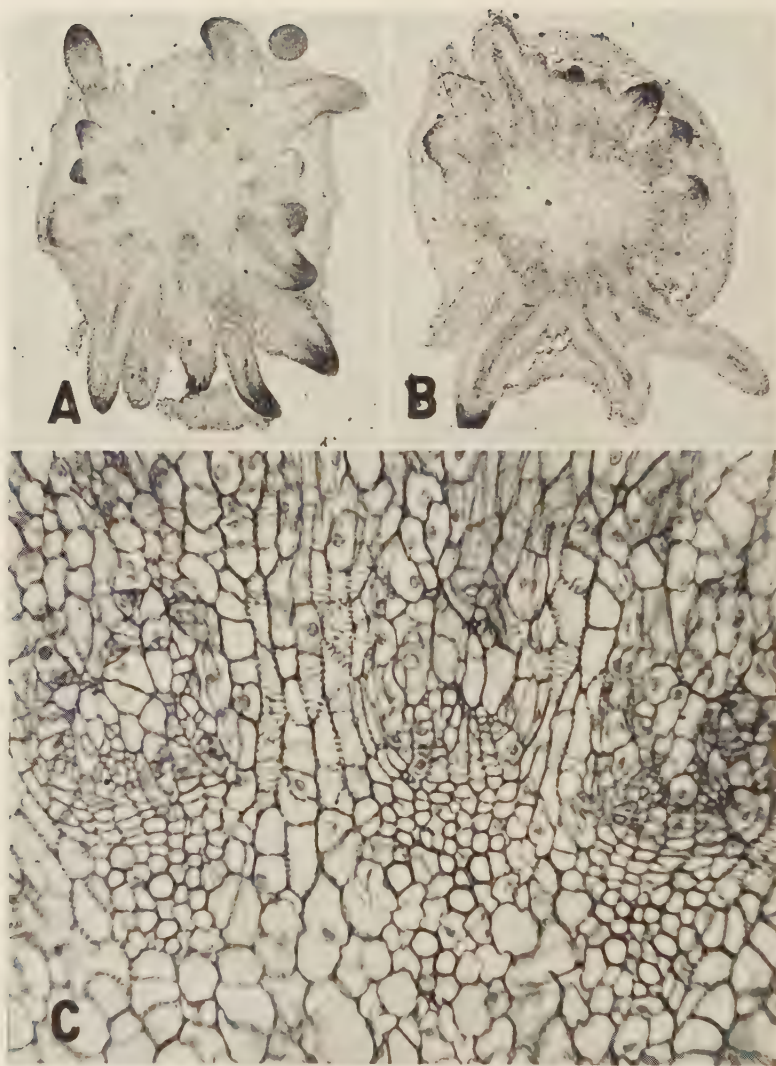


FIG. 10.—*A*: laterally treated first internode 312 hours after treatment. Roots lifting cortex free of stem in places; bundles still rather distinct; pith scarcely affected. *B*: apically treated first internode 312 hours after treatment. Pith rays more reactive than in *A*, giving rise to a broader and more nearly continuous band of meristematic tissue; bundles much less distinct; cortex less affected. *C*: details of region of bundles 312 hours after apical treatment. Numerous tracheids are differentiated; not all of them lead to roots. Phloem very active, ray tissue very active but activity does not extend far into pith (*cf.* fig. 6*B*).

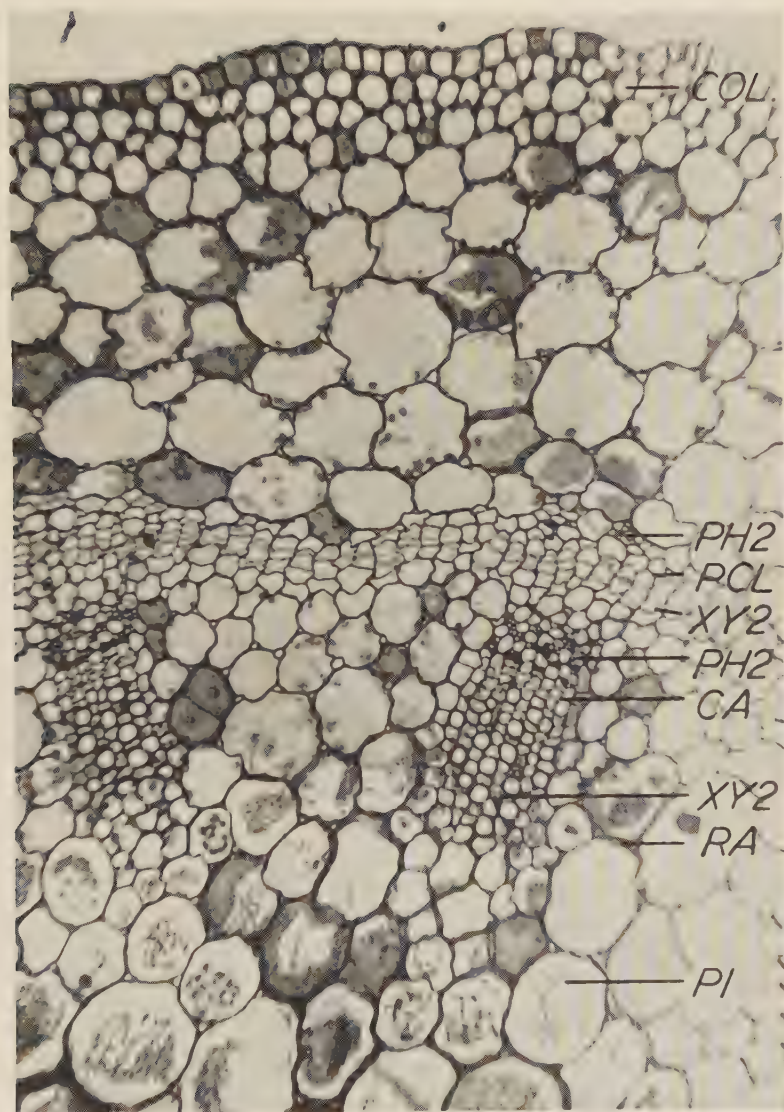


FIG. 11.—Transection of normal third internode at beginning of experiments, showing tissues and stage of development (*col*, collenchyma; *pcl*, pericycle; *ph₂*, secondary phloem; *xy₂*, secondary xylem; *ph₂*, secondary phloem of bundle; *ca*, cambium; *xy₂*, secondary xylem of bundle; *ra*, ray; *pi*, pith).

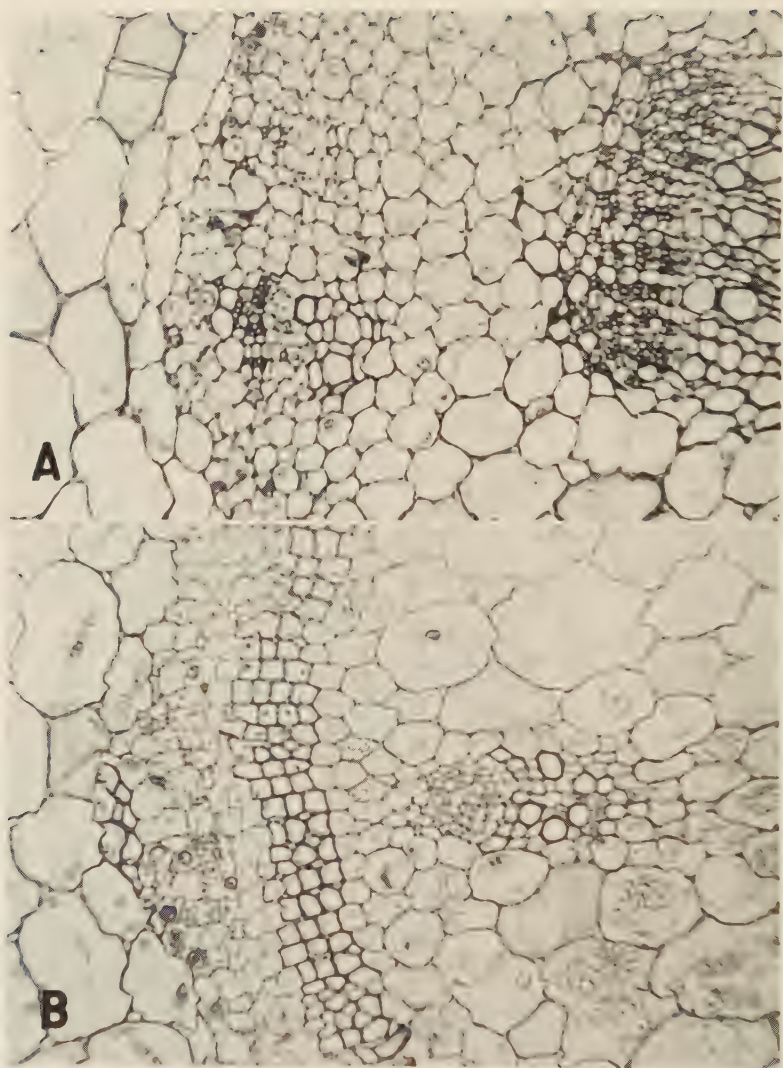


FIG. 12.—*A*: third internode 48 hours after lateral treatment, showing phloem of outer bundle and other derivatives of pericycle beginning to proliferate. *B*: eight hours after treatment; pericycle and its unthickened derivatives outside of thickened secondary xylem cells have become very active; tissues inside this ring not affected.

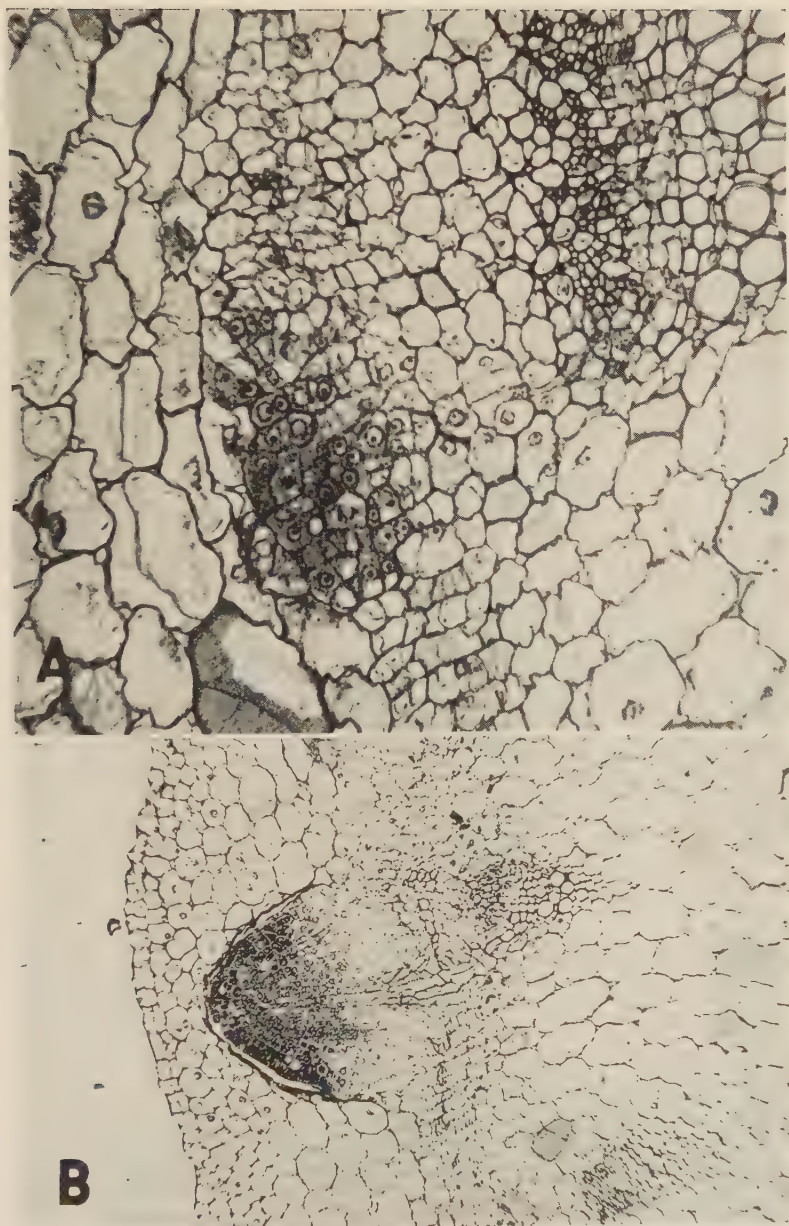


FIG. 13.—Third internodes in which the conjunctive tissue had not thickened at time of treatment. *A*: root primordium arising from pericycle and its derivatives; cells inward to primary bundle becoming involved. *B*: older root primordium, 216 hours after treatment, from which vascular tissue is differentiating back to primary bundle but also back to secondary bundle. Pericyclic tissue above bundle has proliferated to form a wide band; ray and cortex slightly active; most of epidermal cells have divided tangentially but with little enlargement.

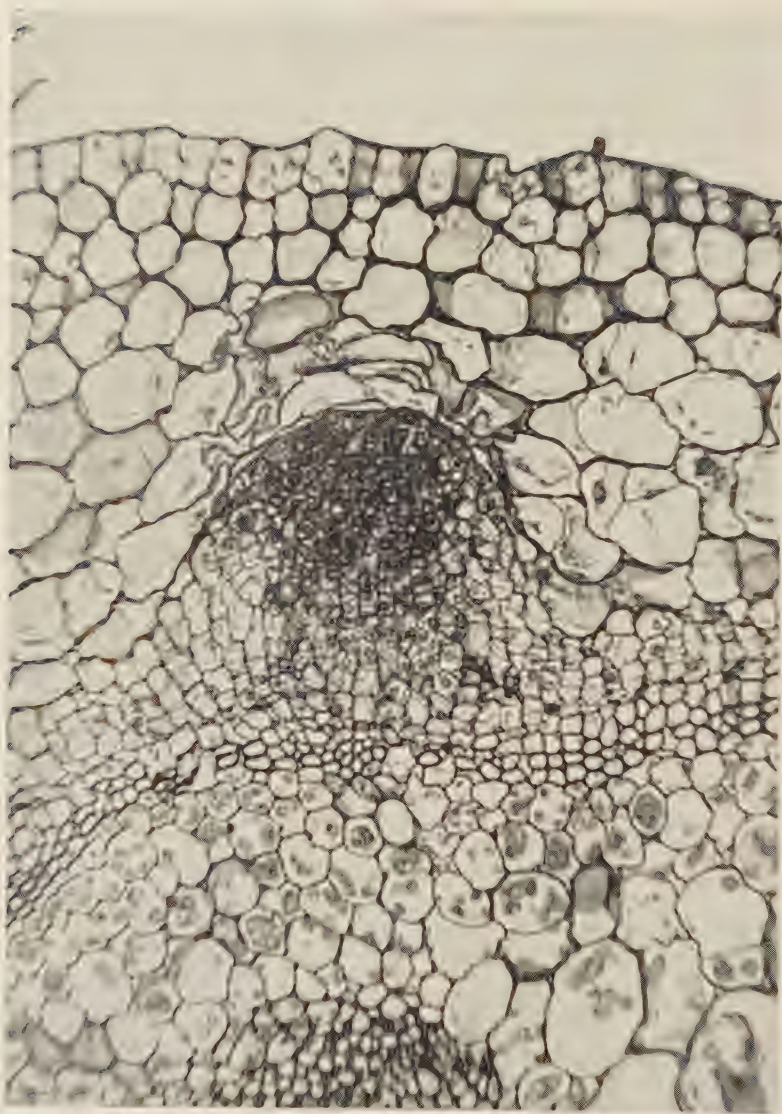


FIG. 14.—Internode 144 hours after treatment; primordium of root which has developed from tissue outside of band of thickened conjunctive tissue. Only epidermis, pericycle, and its derivatives are active.



FIG. 15.—Internode 216 hours after treatment; older root primordium which has developed from tissues outside of thickened band. Vascular tissue of root has differentiated back and tied up with xylem of secondary bundle formed from pericycle. No tissues inside thickened band are involved.

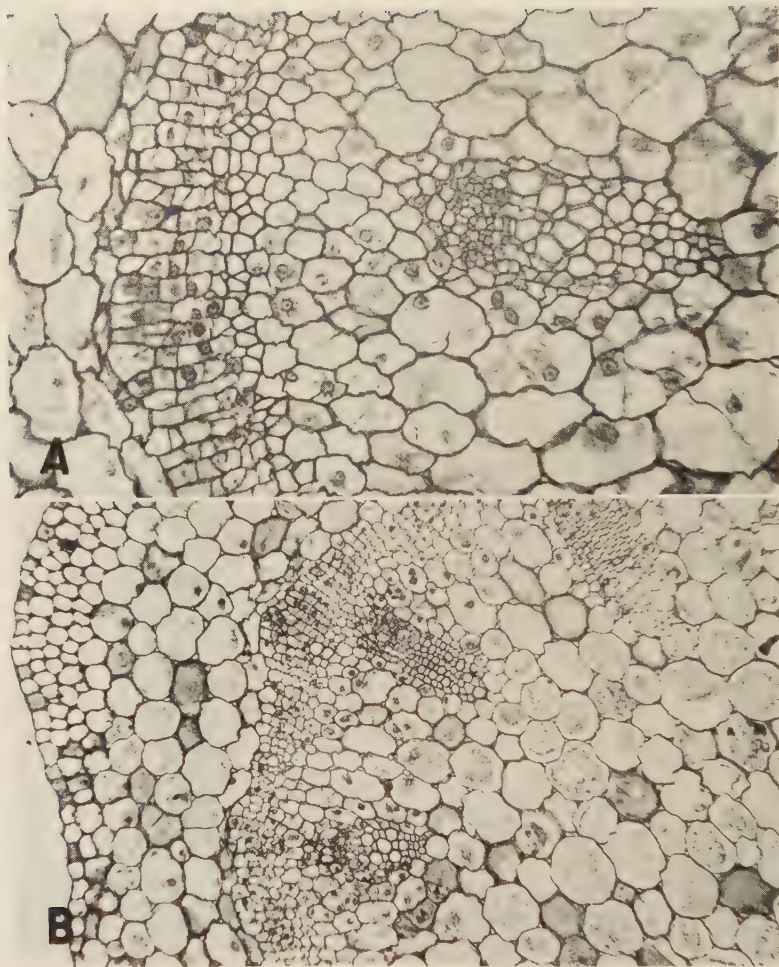


FIG. 16.—Decapitated third internodes apically treated. *A*: 80 hours after treatment pericyclic band has begun to proliferate. *B*: 110 hours after treatment. Phloem of primary bundles, derivatives of cambium, and ray tissue have also become active; root primordium developing in pericyclic zone; other tissues not affected noticeably.

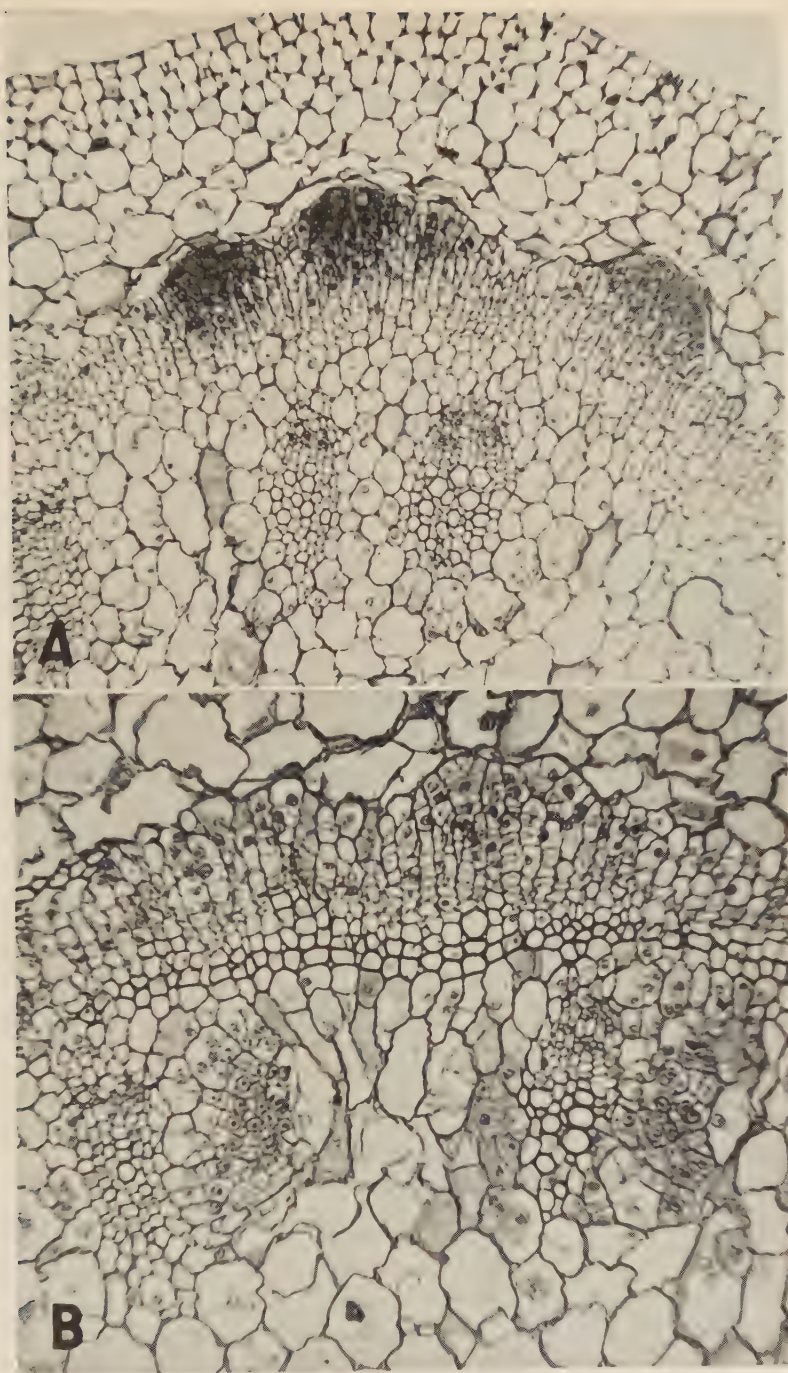


FIG. 17.—Apically treated third internodes. *A*: 144 hours after treatment. Most cells of pericyclic zone are meristematic; three root primordia have developed from this region. Pith cells adjacent to primary xylem of one bundle have divided repeatedly. *B*: 168 hours after treatment. Two root primordia have arisen from ray tissue adjacent to bundles and are growing sidewise instead of outward. Pericyclic derivatives outward from thickened cell are very meristematic.

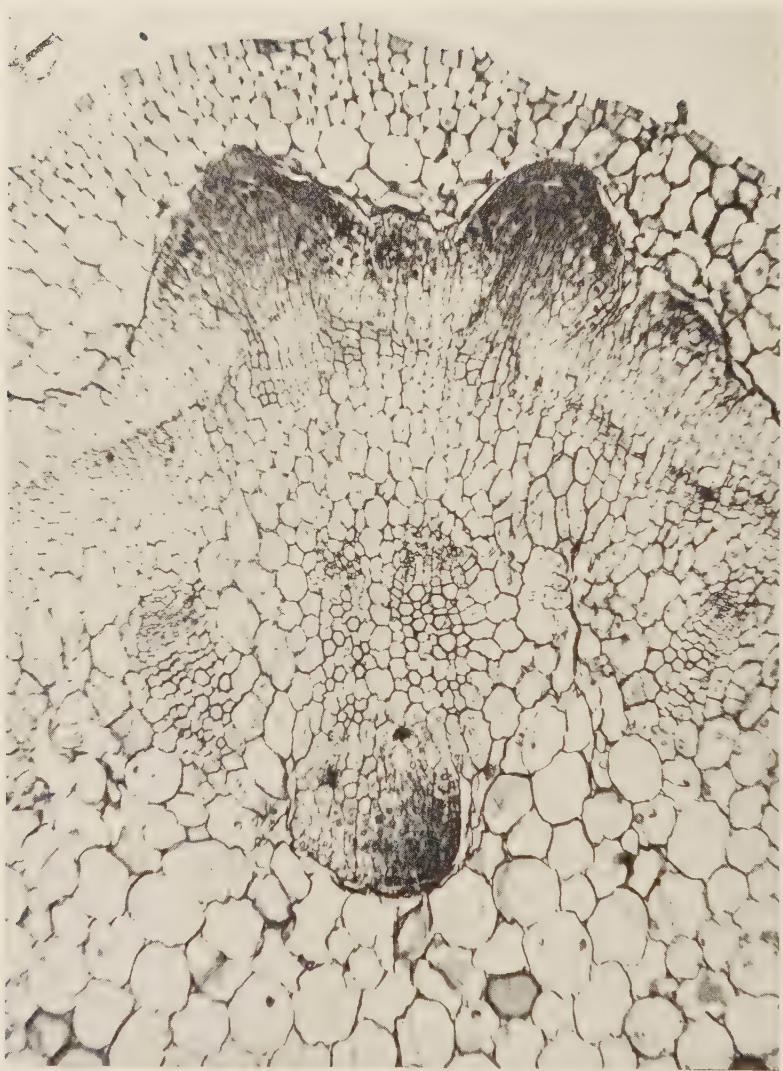


FIG. 18.—Third internode 240 hours after apical treatment. Root primordia developed from pith cells adjacent to primary xylem and growing inward. Xylem parenchyma cells have enlarged, pushing xylem elements apart. Other primordia growing outward from pericyclic zone. Vascular tissue is differentiating back to xylem of outer ring and also to primary bundles. Ray tissue highly meristematic; epidermis and cortex hardly affected at this level, which is about 1.25 mm. below cut surface.

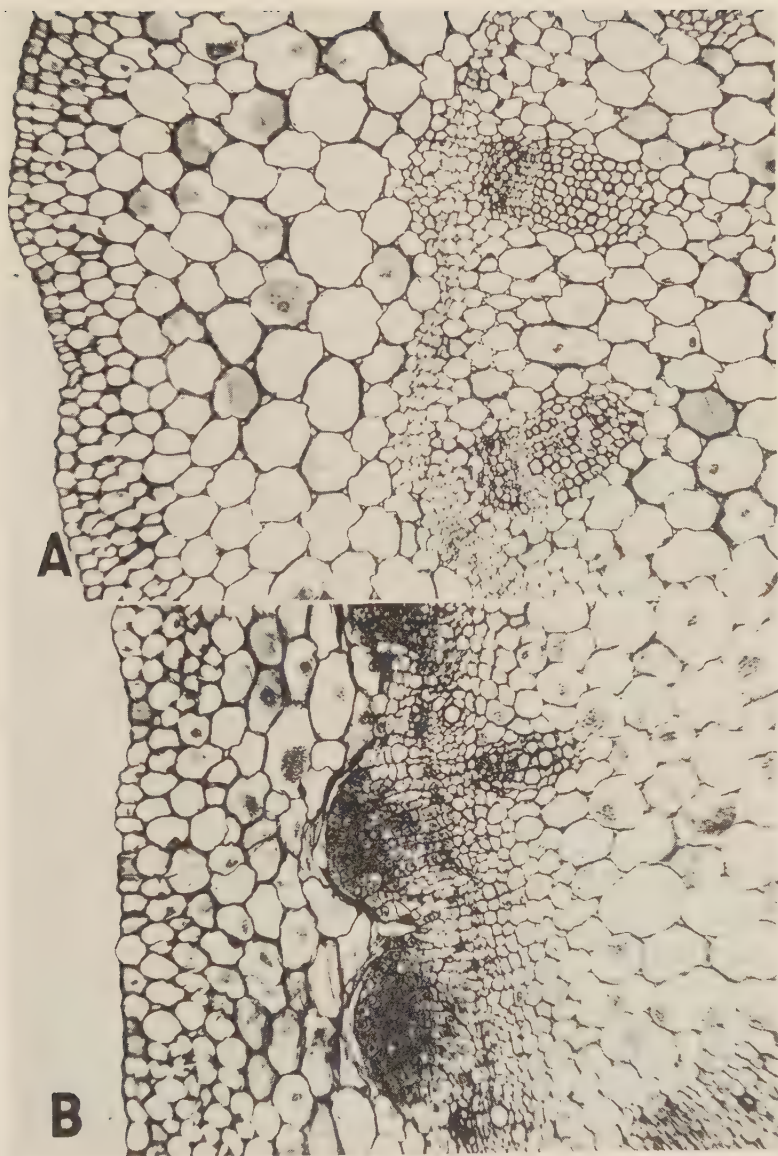


FIG. 19.—*A*: decapitated third internode treated apically with pure lanolin as control, 384 hours after treatment. Cells of epidermis and collenchyma slightly elongated radially; other tissues as in normal stem of this age. *B*: normal rooting; root primordia produced by stem cutting, untreated except for planting in sand; 144 hours after planting; about third or fourth internode.

internal to the ring of conjunctive cells. The cells of the phloem, rays, xylem parenchyma, and the derivatives of the fascicular cambium all proliferated much as in apically treated first internodes. The cells of the pith and of the ray tissue, however, showed even greater activity than did similar tissues in apically treated first internodes. In a number of cases root primordia were organized from proliferating ray cells, inward from thickened conjunctive cells. Such roots were prevented from growing outward by the layers of thickened conjunctive cells (fig. 17*B*).

Of more unusual occurrence was the development of root primordia from cells of the pith, adjacent to the protoxylem (fig. 18). The cells from which these roots developed are some distance from the nearest phloem (figs. 17*A*, 18). A number of such primordia were observed in several different stems.

In decapitated third internode controls which were treated apically with pure lanolin, the epidermal cells enlarged slightly. The cells of the collenchyma enlarged considerably but very few divided. Within the upper 750 μ the cortex and pith became active, the cells dividing in all planes. There was also a slight enlargement of the pith and cortex. Below 1 mm. from the treated surface the tissues appeared about as in an untreated stem (fig. 19*A*).

Discussion

Stems of *Iresine* proved to be extremely sensitive to aqueous solutions of indoleacetic acid; treatments which in other herbaceous plants produce greatly increased rooting were highly toxic to them. Increased growth and cell division took place when the acid was supplied in dilute aqueous solution for a short time, or in lanolin paste from which it diffuses slowly.

The proliferation of cutenized epidermal cells with thickened walls in response to treatments appears to be of rather unusual occurrence, although CROOKS (2) has reported the formation of shoots from the epidermis of flax seedlings following wounding, and the enlargement of the epidermal cells of treated tomato and bean plants has been reported. Whether this response results from the presence of indoleacetic acid is questionable, since stems treated with pure lanolin react in the same way and to about the same degree. Perhaps the

results may be owing in part to the inhibition of gaseous exchange and respiration. Similarly, in stems treated with the lanolin mixture the cells of the collenchyma enlarged and divided, often losing all resemblance to collenchyma; but when treated with pure lanolin such cells enlarged greatly but very few divisions were observed.

The activity of the ray tissue and the phloem in the first internodes is similar to that reported for the epicotyls of beans, but *Iresine* differs sharply from the latter in the lack of pronounced activity of the endodermis, cortex, and pith. No large apical tumors which rise above the treated surfaces are formed in *Iresine*; such proliferation as does occur is limited largely to the first millimeter below the cut surface. While nuclear divisions follow in close succession in the proliferating cells, multinucleate cells as reported for the bean were not observed, although an occasional binucleate cell was found. The general degree of activity of *Iresine* resembles more closely that reported for the tomato than that of the bean. The production of lateral roots is similar in respect to origin, development, and behavior in beans, tomatoes, and the first internodes of *Iresine*. In the third internodes of the latter a different anatomical pattern as a result of secondary thickening is present at the time of treatment. Whereas in the first internodes at time of treatment only primary vascular bundles have been differentiated, in the third internode this series of primary bundles has become surrounded by a meristem from the pericycle and its derivatives. Thus a different anatomical pattern constituting quite a different distribution of cells of greater or lesser degrees of differentiation or maturation results in a somewhat different series of responses to treatment with the lanolin mixture. In laterally treated third internodes the only tissues to respond markedly are those which lie external to the thickened conjunctive tissue. That the indoleacetic acid does not reach the tissues inside this band in sufficiently great concentration to cause them to react seems possible when the behavior of apically treated third internodes is considered. Something more than the concentration of indoleacetic acid must be involved, however, since the quantity which stimulates the pericycle and its derivatives to become highly meristematic must pass through the cortical cells; but they do not respond beyond a comparatively limited enlargement. The sup-

position that the band of thickened conjunctive tissue hinders or prevents the passage of indoleacetic acid beyond its inner limits—and as a result there is an accumulation of the indoleacetic acid in the pericycle and its derivatives which have not become thickened—may be a plausible explanation for the great activity of these tissues, but such an explanation could not account for the varied responses of different tissues when apical treatments to the cut surfaces are made. There appear to be some inherent differences in the capacities of cells of different tissues to respond.

In sharp contrast to the responses to lateral treatments, the tissues within the cylinder of thick walled conjunctive cells became very active following apical treatments. Not only did the cells of these tissues enlarge and divide but some of them gave rise to root primordia. The most noteworthy example of this was the organization of root primordia from cells of the pith. The initiation of roots which grew inwardly following treatment with the lanolin mixture has been reported previously for tomato (1). The internal roots in tomato are reported as arising from the internal phloem. It has been suggested that growth-promoting substances are transported in the phloem, and this may be true in the case of tomato. In *Iresine*, however, the internal roots developed from pith cells located near the protoxylem and at some distance from the nearest phloem. It is unlikely that the cells which initiated these roots were stimulated directly by indoleacetic acid which was transported in the phloem. It is more feasible to assume that they were stimulated by indoleacetic acid which passed down the adjacent xylem elements. This tends to support the suggestion of HAMNER and KRAUS (5) that “apparently some of the indoleacetic acid may travel in vessels in the xylem.”

In stems of the age used in this investigation the fascicular cambium is relatively active. There is, however, no marked increase in the activity of this cambium following treatment with indoleacetic acid. It appeared to maintain about the same relative rate of division as in untreated stems. This is in contrast to the condition reported for *Helianthus* (9), for *Coleus* (4), and for bean (6). It is also noteworthy that in laterally treated stems the collenchyma

cells with heavily thickened areas become more markedly meristematic than do the parenchymatous cells of the cortex.

A rational explanation for the variations in reactivity of different tissues of different plants must await further detailed information on the movement of the various growth substances in plant tissues and the conditions under which their effects on the various cell constituents become manifest. The relative degree of maturation of the tissues and the cells, the external environment, and the variations in the so-called correlative factors at the time of treatment must all play a part in the rate and extent of the responses exhibited.

Summary

1. The rooting of stem cuttings of *Iresine lindenii* treated with weak aqueous solutions of indoleacetic acid for a short time was hastened and the number of roots increased.

2. First and third internodes were each treated with 3% indoleacetic acid in lanolin applied laterally as a ring or apically to decapitated stems. Similar internodes were treated with pure lanolin as controls.

3. The gross responses of the plants were observed over a period of two to four weeks. Tumors were formed and numerous adventitious roots developed in the treated areas.

4. In the older internodes an extrafascicular cambium derived from the pericycle gives rise to a band of conjunctive tissue and secondary vascular bundles. Differences in the responses of first and third internodes may be correlated with this structural feature.

5. Histological studies showed that all living tissues of the stem react to some extent to treatments with indoleacetic acid. The cells of the pericycle and its derivatives, rays, and phloem were the most generally responsive, although the epidermis and collenchyma became very active in laterally treated stems. Neither the cambium nor the endodermis was markedly stimulated.

6. Lateral roots developed from the tissue of the ray, phloem, and pericycle in first internodes. In third internodes roots external to the conjunctive tissue developed from the pericycle and its derivatives and from the phloem of secondary bundles. Such roots as

were formed internal to the conjunctive tissue developed from the ray tissue and from the pith.

7. The responses of treated *Iresine* stems were compared with those of other plants and some of the implications are discussed.

The writer is indebted to Dr. E. J. KRAUS and Dr. C. A. SHULL for suggesting this problem and for their very generous help during the progress of the investigation.

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